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Exploring the Antibacterial Potential of Alcoholic Ginger Extract Against Key Gram-Negative Pathogens: *E. Coli* and *Pseudomonas Aeruginosa*

Zahraa Basem*

Department of Medical Laboratory Techniques, College of Health and Medical Techniques /kufa, Al Furat Al Awsat Technical University

ABSTRACT

Ginger, scientifically known as Zingiber officinale, is a popular spice and medicinal plant used in various traditional and alternative medicine practices. While ginger has many potential health benefits, including anti-inflammatory and antioxidant properties, its effectiveness as an antibacterial agent is still a subject of ongoing research and debate**.** This study was conducted in order to determine the antibacterial activity of alcoholic ginger extract on some Gram-negative bacter. Obtaining ginger from the local market in Najaf City - Najaf Governorate – Iraq and bacterial isolates *Pseudomonas aeruginosa and Escherichia coli* Obtained From the Department of Biology- College of Science - University of Kufa and confirmed by cultural and biochemical examination and ultimately by VITEK-2 compact system . Four types of antibiotics were used to find out the bacteria's resistance to these antibiotics, which are Meropenem, Imipenem , Ciprofloxacin and Gentamycin. The results showed *Pseudomonas aeruginosa* resistance to all antibiotics except for ciprofloxacin, which was sensitive to it, while *Escherichia coli* was sensitive to all antibiotics. Antibacterial activity of alcoholic ginger extract on *Pseudomonas aeruginosa and Escherichia coli* showed the inhibition zone , where the diameters of inhibition reached(20-16-14-11-0) mm for *Pseudomonas aeruginosa* while The diameters of the inhibition zone were (18-15-13-11-0) mm for *Escherichia coli* at the concentrations (1000,800,600,400,200) ug/ml, respectively, compared to the control group, which was not effective. The results of study indicate to the concentration 1000 ug/ml more effective on both types of bacteria from another concentrations.

KEYWORDS: Antibacterial**,** *Pseudomonas aeruginosa***,** Ciprofloxacin, Gentamicin**.**

INTRODUCTION

Ginger (Zingiber officinale) has been one of the most commonly used herbal medicines to treat several prevalent diseases for a very long time. Ginger contains antibacterial activity, antioxidant properties, and numerous bioactive compounds, which could be used as an alternative treatment for numerous infectious diseases (**1**). In the past, it has been shown that the active ingredients in ginger prevent the growth of digestive bacteria. Ginger has potent antimicrobial characteristics that can kill or slow the growth of harmful bacteria (**2**).

Before the discovery of antibiotics, aromatic herbs and medicinal plants were the primary treatment for infectious diseases (**3**). Microorganisms have developed resistance factors as a result of the increased use of antibiotics, which has created a dire situation (4).

Escherichia coli is typically an animal and human commensal. Intestinal and extra-intestinal illnesses brought on by pathogenic variations include gastroenteritis, urinary tract infections, meningitis, peritonitis, and septicemia (**5**). Humans are frequently exposed to antimicrobial substances, which has a significant impact on the spread of antimicrobial resistance and the emergence of bacteria that are resistant to antimicrobials. For instance, Escherichia coli isolates found in animals treated with antibiotics can infect humans. Human illnesses and outbreaks have been associated with higher plantbased diet consumption (**6**). *Pseudomonas aeruginosa* is a non-sporing, non-capsulated, gramnegative bacillus. It can affect almost any external site or organ. Infection in hospital includes urinary tract infection, infected ulcers, burns and eye infections. (**7**).Due to *Pseudomonas aeruginosa's* capacity to withstand several of the currently available medicines, treating infections caused by this bacterium has become extremely difficult(**8**).

The development of new antibiotics to treat infections is urgently needed for three bacterial species, according to the world's health organisation (WHO), including carbapenem-resistant Pseudomonas aeruginosa (**9**).

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MATERIALS AND METHODS

MATERIALS:

Ginger: Obtaining ginger from the local market in Najaf City - Najaf Governorate – Iraq,Bacterial isolates: Bacterial isolates *Pseudomonas aeruginosa* and *Escherichia coli* Obtained From the Department of Biology- College of Science - University of Kufa and confirmed By cultural and biochemical examination(10-11) and ultimately by VITEK-2 compact system,Chemical and cultural material as Ethanol alcohol and MacConcky agar and Muller Hinton agar and Nutrient agar Normal Saline and some antibiotic disc and Dimethyl Sulfoxide.

METHODS

Preparation and extraction of ginger

The first step for extraction involved the preparation of dry powder from ginger rhizomes. For this, fresh gingers were brought from the local market in Najaf City - Najaf Governorate – Iraq. The gingers were then thoroughly washed. After washing, they were peeled, sliced into pieces and then dried by an electric oven for 5 days to remove the moisture. Removing of sufficient moisture content of crude drugs was necessary to improve its quality and make it resistant to the growth of microorganisms. The dried ginger samples were then powered by mechanical grinder and sieved to give powdery form. The powder was stored in polythene bag at room temperature before extraction. The Soxhlet apparatus was used according to the method mentioned by (**12**). A weight of 10 g of dried vegetable powder was added to it, 250 ml of 96% ethanolic alcohol was added to it, and it was turned on for 12 hours, then the extract was dried by an electric oven for a week at a temperature 42 C. Then, a stock solution was prepared by taking 1 mg, 0. 8 mg, and 0. 6 mg , 0. 4 mg 0.2 mg of ginger extract powder to dissolve them in 1 ml of solvent to prepare concentrations (1000,800,600,400,200) ug/ml, respectively, and the solvent used was consist of Dimethyl Sulfoxide, (DMSO) then filter the storage solution for sterilization with special filters Whatman membrane filter (0.22) µm(**13**).

Prepartion of culture media

Media used in this study prepared accordance to the manufacturer's instructions fixed on their containers. All the media were sterilized by autoclaving at 121°C, for 15 min., then poured into sterile Petri dishes

Inoculum preparation

The method mentioned by (**14**) was used to prepare the bacterial inoculum by growing the activated bacteria on Nutrient Agar medium and incubating it at 37 degrees for 24 hours, then transferring ten colonies of each type of bacteria used in the experiment and under sterilization conditions to a test tube containing on 5 ml of Nutrient Broth and then incubated at 37 °C for (4-6) hours

Antibiotic Susceptibility Test

For this test, the fresh inoculums of test microorganism were taken. two sterile petri plates were taken and Mueller Hinton agar was poured to it and allowed to solidify at room temperature. Sterile Mueller Hinton agar plate was uni-formly swabbed with a specific bacterial culture. Then, four different antibiotics discs namely Ciprofloxacin, Gentamycin ,Meropenem and Imipenem having respective concentrations of 5µg, 10µg , 10µg and 10µg were placed. The plates were incubated at 37oC for 24 hours (**14).**

Study the effect of ginger alcoholic extract on the growth and activity of bacterial species

Minimum inhibitory concentration by using Agar well diffusion method was used to observe the effect of the extract on the growth of the isolated bacteria under study. Spread 0.1 ml of the bacterial inoculums on the surface of The Muller Hinton agar then the dishes were left on the medium at room temperature for 15 minutes to allow for absorbing the inoculum, then work 5 wells with 10 mm diameter by cork borer and then added 100 µl of each concentration of the plant extracts under study in 4 well, and At the same time 100 µl of sterile distil water was placed in last well as control instead of the plant extract by using micropipette, and the addition must be on the surface of the culture media carefully, then the petri dishes were incubated in 37°C for 24hr. (15).

RESULTS AND DISCUSSION

In the study of antibiotic Susceptibility Test of four different types of antibiotics on *Pseudomonas aeruginosa* and *Escherichia coli* , the results appeared clearly *Pseudomonas aeruginosa* have resistance against Gentamycin ,Meropenem and Imipenem while have against Ciprofloxacin on another hand *Escherichia coli* have sensitive against all Meropenem ,Imipenem , Ciprofloxacin and Gentamycin this results can showed clearly in table (1) below

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Table: (1) The inhibition zones of standard antibiotics disc against *Pseudomonas aeruginosa and Escherichia coli*

Bacteria	**Diameter of inhibition zone for antibiotics disc (mm)**			
	Meropenem	Imipenem	Gentamycin	Ciprofloxacin
Pseudomonas aeruginosa	**0**	**13**	**0**	**28**
Escherichia coli	**28**	**26**	**22**	**28**

According to table (1) that refer to the results of antibiotic Susceptibility Test of *Pseudomonas aeruginosa* near to the results of study recorded by (**16**) The results showed a high resistance to Imipenem, and on the other hand, a high sensitivity to an Ciprofloxacin and another study recorded by (**17**) The results showed a high resistance to Meropenem and Gentamycin.The results shown in the table(1) for *Escherichia coli* are similar to the results recorded by (**18**) which indicate a high sensitivity of the bacteria to all antibiotics used ,Another study recorded by (**19**) shows similar results. In the study of the effect of agar well diffusion plate assay method, the results appeared clearly the effect the ethanolic extract of ginger was highly effective against the bacteria used in the study compared with the control group, this results can showed clearly in table (2) and figure (1).



Figure: (1) The antibacterial effect of alcoholic extract of ginger against *Pseudomonas aeruginosa and Escherichia coli* by agar well diffusion method (10 mm).

Table: (2) The inhibition zones of alcoholic extract of ginger against *Pseudomonas aeruginosa and Escherichia coli*

Bacteria	**Diameter of inhibition zone for each concentration (mm)**					
	200 ug/ml	400 ug/ml	600 ug/ml	800 ug/ml	1000 ug/ml	Control
Pseudomonas aeruginosa	**0**	**11**	**14**	**16**	**20**	**0**
Escherichia coli	**0**	**11**	**13**	**15**	**18**	**0**

Through the results that appear to us in Table (2), we note that concentration 200 ug/ml of the alcoholic extract of ginger does not have inhibitory activity against any of the two types of bacteria used in the study, while concentration 400 ug/ml has an inhibitory effect equally towards both types estimated at 11 mm in diameter , while in concentrations 600 ,800 And 1000 ug/ml, the inhibitory activity against *Pseudomonas aeruginosa* is 14 16, and 20, respectively, and the inhibitory effectiveness against *Escherichia coli* is 13, 15, and 18 respectively, Hence, we notice something that must be studied extensively, as although *Pseudomonas aeruginosa* are resistant to antibiotics, they are more affected by the extract than *Escherichia coli*. these concentrations compared with well contain distill water as control. The results of study indicate to the concentration 1000 ug/ml more effective on both types of bacteria from another concentrations.The results in this study similar to recorded by (**20**) that show effect of ginger alcoholic extract on *Escherichia coli* in addition to another types of bacteria .

Another study presented by (**21**) indicates almost the same results obtained in this study, especially the concentration of 1000 ug/ml, which showed a clear effect on *Pseudomonas aeruginosa and Escherichia coli* . Another study also show the same results recorded by (**22**) refer to ginger alcoholic extract have effect against bacteria under study.

CONCLUSIONS AND RECOMMENDATIONS

1-The ethanolic extract of ginger has the ability to inhibit the two types of bacteria under study
2- Study of the biological activity of ginger and its inclusion in the manufacture of antibiotics instead of chemicals
3-Using the aqueous extract and using other types of bacteria to find out the extent of their effect on the bacteria targeted in the study.
4- Analytical studies to find out the active compound in ginger to used it against microorganism
5- Giving information to people about the importance of using ginger in meals because of its great benefit
6- A comparative study between the effect of ginger and turmeric on different types of bacteria and fungi

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